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During the course of the research studies were made of the following: Analysis of S. aurantia motility, isolation and characterization of chemotaxis (che) mutants, S. aurantia flagella, cloning of genes encoding S. aurantia flagellar polypeptides, cloning a gene that complements E. coli trpE mutants, S. aurantia. Results of these studies appear in the final report.

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1. Greenberg, E. P., B. Brahamsha, and K. Fosnaugh. 1985. The motile behavior of *Spirochaeta aurantia*: A twist to chemosensory transduction in bacteria, pp. 107-118. In: Sensing and response in microorganisms (M. Eisenbach and M. Balban, eds.). Elsevier.
2. Kropinski, A. M., T. R. Parr, Jr., B. L. Angus, R. E. W. Hancock, W. C. Ghiorse, and E. P. Greenberg. 1987. Isolation of the outer membrane and characterization of the major outer membrane protein from *Spirochaeta aurantia*. J. Bacteriol. 169:172-179.
3. Brahamsha, B., and E. P. Greenberg. 1987. Complementation of a *trpE* deletion in *Escherichia coli* by *Spirochaeta aurantia* DNA encoding anthranilate synthetase component I activity. J. Bacteriol. 169:3764-3769.
4. Fosnaugh, K., and E. P. Greenberg. 1988. Motility and chemotaxis of *Spirochaeta aurantia*: Computer-assisted motion analysis. J. Bacteriol. 170:1768-1774.
5. Brahamsha, B., and E. P. Greenberg. 1988. Structure of and chemical analysis of periplasmic flagella from *Spirochaeta aurantia*. J. Bacteriol. (In press).
6. Fosnaugh, K., and E. P. Greenberg. 1988. *Spirochaeta aurantia* chemotaxis mutants: A behavioral and biochemical analysis. (Submitted for Publication).

8. SCIENTIFIC PERSONNEL SUPPORTED BY THIS PROJECT AND DEGREES AWARDED:

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PROGRESS REPORT

Analysis of *S. aurantia* motility. In an article published in 1985 (E. P. Greenberg, B. Brahamsha and K. Fosnaugh) we summarized most of the available information on spirochete chemotaxis and we described a crude analysis of *S. aurantia* motility (reprint included in appendix). Based on the crude analysis we postulated that *S. aurantia* performs three distinct behaviors; runs of smooth swimming, reversals and flexes. According to the model described, runs occur when the two flagella motors are coordinated in a CW-CCW mode, reversals occur when both motors switch orientation in synchrony and flexing occurs when the flagellar motors are in a CW-CW or CCW-CCW mode.

More recently we developed a computer program for quantitation of behavior in populations of *S. aurantia* cells and we used this program to analyze changes in behavior in response to chemoattractants (K. Fosnaugh and E. P. Greenberg, 1980). Based on the data collected with this program, we presented a model to explain the behavior of *S. aurantia* and the response of cells to chemoattractants. We have used the newly developed computer-assisted automated motion analysis to study the behavior of chemotaxis mutants (see below) and we plan to use this capability to study chemorepellent responses.

Isolation and characterization of chemotaxis (*che*) mutants. Five classes of *che* mutants have been isolated. Class I mutants exhibit running and flexing but do not reverse, Class II mutants flex incessantly. Class III mutants reverse at an extremely high frequency (2-3 times that of wild-type), and the Class IV and V mutants exhibit unstimulated behavior similar to the

wild-type. All of the mutants showed normal MCP methylation and they all exhibited membrane potential responses to chemoattractants that were distinct from wild-type (K. Fosnaugh and E. P. Greenberg, see enclosed preprint).

The mutant analysis supports the hypothesis that chemotaxis in *S. aurantia* involves electrogenic signaling. Furthermore, the isolation of mutants incapable of reversals and those which reverse too often demonstrates there is a genetic and biochemical basis for reversing, that reversals do involve a switch synchronizing device and that they are not just a coincidental behavior.

The *S. aurantia* flagella. We have purified flagella from *S. aurantia* by a procedure involving Triton X-100 and KBr gradient centrifugation. The hook basal body (HBB) complex consists of a set of two rings, a rod and a hook. There is a proximal button-like structure similar to that observed with *Caulobacter* flagella. The flagellar filament is complex in that an inner core and an outer layer can be observed by electron microscopy. To date we have identified 9 proteins which at least in part constitute the flagella. Three proteins in the 60-65 kdaltons range predominate in preparations consisting of HBBs (the filaments can be solubilized by acid treatment) and thus, these proteins have been assigned as HBB components. Six proteins in the 30-38 kdalton range occur as components of the flagellar filament. By analyzing a mutant of *S. aurantia* which possesses hooks and basal bodies but no filaments we have demonstrated that all of the six proteins are components of the flagella and not proteins which aggregate with and

contaminate flagella during purification. Using a Western blot analysis with antiserum against flagellar filaments we showed that none of the six polypeptides were present in the mutant (B. Brahamsha and E. P. Greenberg, J. Bacteriol. In press, Preprint enclosed).

Monoclonal antibodies specific for various of the filament polypeptides were produced and used in immunogold labeling experiments to establish a correspondence between structural components and polypeptides. One of the polypeptides (the 37.5 K) is associated with the outer layer or at least the surface of filaments. The other five polypeptides are antigenically related and the results of the gold-labeling experiments led to the conclusion that these are core components (B. Brahamsha and E. P. Greenberg. J. Bacteriol. In Press, Preprint enclosed).

These studies of the biochemistry and structure of *S. aurantia* flagella are important for two reasons: To understand the nature of the propulsive mechanism in *S. aurantia* in detail; to understand the operation of periplasmic flagella as opposed to flagella of other bacteria, this information is crucial. Second we have used our knowledge of the flagella and our antibodies to obtain cloned *S. aurantia* flagella genes. This, in fact, provides an opening to a molecular genetic analysis of spirochete motility and chemotaxis.

Cloning of genes encoding *S. aurantia* flagellar polypeptides. There is little available information on the genetics of any spirochete. Some spirochete genes have been cloned, these include *E. coli trpE* complementing genes from *Leptospira* and from *S. aurantia* (B. Bahamsha and E. P. Greenberg, 1987, see reprint enclosed in Appendix), and a variety of surface antigens from pathogenic spirochetes such as *Treponema pallidum*, the causative agent of

syphilis. With the exception of our analysis of the *S. auranita trpE* gene (in collaboration with Prof. Irving Crawford, University of Iowa), there is virtually nothing known about gene organization in any spirochete (the analysis of *trpE* is discussed below).

To initiate our analysis of the molecular basis of *S. aurantia* motility and chemotaxis we created an *S. aurantia* gene library in the expression vector, pUC18. This vector was chosen for several reasons: The G+C content of *S. aurantia* is above 60 mols%. Genes from other high G+C organisms are poorly expressed in *E. coli*, presumably because such organisms possess promoters that are not A-T-rich as are *E. coli* promoters. Also, spirochete RNA polymerases are distinct from other eubacterial RNA polymerases suggesting the possibility that different RNA polymerase binding sites exist in these organisms.

Our initial interest has centered on cloning of flagellar filament antigens. We have purified flagella from *S. aurantia* and characterized these flagella biochemically and cytologically (see above). Furthermore, we had obtained antiserum against *S. aurantia* flagella and monoclonal antibodies against specific polypeptide components of *S. aurantia* flagellar filaments (as discussed above). By colony-blotting procedures, several clones of *E. coli* which produced *S. aurantia* flagellar antigens were obtained. All of the clones were studied using Western-blot procedures with the polyclonal antiserum and with the monoclonal antibodies as probes. We are now in the process of sequencing the *S. aurantia* DNA from the most straight-forward of our clones. This clone produces a 37.5 kilodalton protein which reacts with the monoclonal antibody specific for the 37.5 kilodalton flagellar filament protein (B. Brahamsha and E. P. Greenberg, In preparation).

Cloning a gene that complements *E. coli trpE* mutants. In an initial publication (B. Brahamsha and E. P. Greenberg, 1987) we described the cloning of a *S. aurantia* DNA fragment that complemented an *E. coli trpE* deletion and we demonstrated that the *S. aurantia* DNA encoded an anthranilate synthetase component I which was capable of interaction with the *E. coli* component II. The interaction with an *E. coli* protein to yield a functional anthranilate synthetase led to the hypothesis that *S. aurantia* motility and chemotaxis gene products might be able to interact with *E. coli* motility and chemotaxis proteins thus complementing *E. coli* motility and chemotaxis mutations. However, due to the high frequency of second-site (suppressor) mutations of the *E. coli* mutants the hypothesis could not be adequately tested. That is we were unable to obtain cloned motility and chemotaxis genes by screening transformed *E. coli* mutants although we did obtain many *E. coli* suppressor mutants.

In conjunction with Dr. Irving Crawford at the University of Iowa, we have sequenced through the *S. aurantia trpE* gene and the sequence data extend several hundred base pairs past the open-reading frame in both directions (sequence enclosed with appendixed materials). It is clear from the available data that the *S. aurantia trpE* promoter bears no relation to the standard -10, -35, *E. coli* type. Furthermore, it is difficult to find a ribosome binding site suitable for *E. coli* ribosomes and the downstream sequence is predicted to have extensive secondary structure. Research on *S. aurantia trp* genes has taken on a life of its own and will continue, separate from this project, mainly in Dr. Crawford's lab. The immediate interests are in identifying *S. aurantia* promoters and ribosome binding sites and in developing a genetic transfer system based on homologous recombination.

Ion channels. During a sabbatic leave in my laboratory, Dr. Andrew Kropinski put considerable effort into separating the cytoplasmic and outer membranes of *S. aurantia*. In the course of his studies, we learned that unlike *E. coli* and other Gram-negative bacteria, *S. aurantia* does not contain a classical lipopolysaccharide in the outer membrane. Unfortunately, the presence of lipopolysaccharide in the outer membrane forms the basis for separation of the two membranes of many Gram-negative bacteria. Thus, the standard procedure was not effective with *S. aurantia*.

Dr. Kropinski was successful in isolating the *S. aurantia* outer membrane. The isolation procedure was based on the detergent insolubility of outer membrane compared to cytoplasmic membrane. The most abundant ion channel in *S. aurantia* outer membranes was characterized by single channel conductance measurements in black lipid membranes. This *S. aurantia* channel had many characteristics similar to porins of Gram-negative bacteria, in fact, we consider that this represents a spirochete porin. This spirochete porin is unique in its conductance properties in that it has a higher conductance capacity than any other porin studied with a black lipid membrane technique and in that single channel conductance increases with decreasing salt concentration. For a more complete description of the properties of this porin, see the appendixed article (A. M. Kropinski et al., 1987).

Negative chemotaxis in *S. aurantia*. For many years studies of chemotactic mechanisms in *S. aurantia* have been limited by our inability to demonstrate a chemorepellent response. Thus, some very basic and simple questions could not be addressed. For example, if attractants cause decreased flexing and reversals, do repellents increase flexing and reversals? If attractants cause

a transient membrane depolarization, do repellents cause a hyperpolarization? Are there mutants that cannot respond to attractants but can respond to repellants? The answers to such questions will lead to a better understanding of cellular events that are causally related to chemotaxis vrs. those casually related.

Recently, Dr. Charlotte Kämpf in my laboratory developed a repellent-gradient tube assay for negative chemotaxis in *S. aurantia*. Using this assay she has demonstrated that acids, alcohols and sulfide are effective chemorepellents (see Abstract in the Appendix). Competition experiments indicate the existence of at least three repellent receptors; an acid receptor, and alcohol receptor and a sulfide receptor.

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